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ORIGINAL ARTICLE

The *SREBF-1* locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population

TK Felder[1], H Oberkofler[1], R Weitgasser[2], V Mackevics[3], F Krempler[4], B Paulweber[2] and W Patsch[1]

[1]*Department of Laboratory Medicine, Paracelsus Private Medical University, Salzburg, Austria;* [2]*Department of Internal Medicine, Paracelsus Medical University, Salzburg, Austria;* [3]*Department of Internal Medicine, Riga Stradins University, Riga, Latvia and* [4]*Department of Internal Medicine, Krankenhaus, Hallein, Hallein, Austria*

Context: The sterol regulatory element-binding protein-1c (SREBP-1c) is a transcription factor involved in the regulation of lipid and glucose metabolism and has been implicated in the pathophysiology of type 2 diabetes mellitus (T2DM).
Objective: We aimed to confirm associations of the *SREBF-1* gene with T2DM in an Austrian population and to study possible associations with diabetes-related quantitative traits.
Design, settings and participants: We genotyped a diabetic cohort ($n=446$) along with a control group ($n=1524$) for a common C/G variation that is located in exon 18c (rs2297508) and has been associated with obesity and T2DM in French populations.
Main outcome measures: Body mass index (BMI), indices of insulin sensitivity and β-cell function, plasma adiponectin, T2DM and single-nucleotide polymorphism rs2297508.
Results: Genotype distributions associated with rs2297508 differed by T2DM status ($P=0.0045$), but not by BMI. The variant G allele was associated with a modest, but significant, increase in the prevalence of T2DM after adjustment for age, sex and BMI (G/G: odds ratios (OR) (95% confidence intervals) = 1.45 (0.99–2.11) and G/C: OR = 1.37 (1.04–1.81)). In a cross-sectional population of non-diabetic subjects, associations of rs2297508 genotypes with plasma adiponectin levels adjusted for age, sex and BMI ($P=0.0017$) were observed in that the risk G/G genotype displayed the lowest adiponectin levels.
Conclusions: We observed associations of rs2297508 with T2DM prevalence and plasma adiponectin. SREBP-1c has been implicated in the regulation of adiponectin gene expression. Our results therefore raise the possibility that sequence variations at the *SREBF-1* gene locus might contribute to T2DM risk, at least in part, by altering circulating adiponectin levels.
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Keywords: SREBP-1c; adiponectin; type 2 diabetes; single nucleotide polymorphism

Introduction

The incidence of non-insulin-dependent type 2 diabetes mellitus (T2DM) is increasing world wide at an alarming rate in progressively younger populations. Pathophysiologically characterized by insulin resistance, pancreatic β-cell dysfunction and enhanced hepatic gluconeogenesis, the exact etiology of type 2 diabetes is not known, but obesity and alterations in genetic programs that control glucose and fatty-acid metabolism are key contributing factors.[1]

Correspondence: Dr H Oberkofler, Department of Laboratory Medicine, Paracelsus Private Medical University, Müllner Hauptstr. 48; A-5020 Salzburg, Austria.
E-mail: h.oberkofler@salk.at
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Sterol regulatory element-binding proteins (SREBPs) are members of the basic helix-loop-helix-leucine zipper (bHLH-LZ) family of transcription factors and activate transcription of genes involved in the metabolism of cholesterol, fatty acids, triglycerides and phospholipids.[2] SREBP-1a and -1c isoforms, both encoded by *SREBF-1*, but generated by alternative promoter usage, are more closely related to the regulation of fatty-acid biosynthesis, whereas the SREBP-2 isoform, encoded by *SREBF-2*, is preferentially involved in the regulation of cholesterol homeostasis.[2] SREBP-1c is the predominant isoform in many human tissues, including liver, skeletal muscle and adipose tissue. Glucose-stimulated hepatic *GLUT2* gene expression as well as insulin effects on hepatic gene expression are mediated by SREBP-1c.[3,4] Hepatic SREBP-1c expression is regulated by nutritional stimuli like glucose, cyclic adenosine monophosphate and

polyunsaturated fatty acids.[2] Insulin also upregulates skeletal muscle *SREBP-1c* gene expression thereby increasing the expression of glycolytic and lipogenic enzyme genes.[5] In pancreatic β-cells, overexpression of *SREBP-1c* is associated with accumulation of triglycerides and impairs insulin secretion at least in part via upregulation of uncoupling protein 2.[6]

SREBF-1 expression has been shown to be reduced in obesity and T2DM in humans.[7,8] A meta-analysis of genome-wide scans in four European populations showed linkage between the 17p11 region, comprising *SREBF-1*, and type 2 diabetes.[9] Meanwhile, associations of an exon 18c single-nucleotide polymorphism (SNP) (rs2297508) within *SREBF-1* with obesity and T2DM in French obese and diabetic cohorts [10] and of an intron 18c SNP with T2DM in a British population sample have been reported.[11] Considering T2DM risk, rs2297508 was the most significantly associated SNP among 12 variant sites in the transcribed region of *SREBF-1* analyzed in the French study. Owing to the high linkage disequilibrium (pairwise $D' > 0.9$, $r^2 = 0.88$, $P < 0.001$; www.hapmap.org) observed between the two variant sites in exon 18c and intron 18c, we focused on SNP rs2297508 and aimed to confirm the association of this sequence variation in the transcribed region of *SREBF-1* with T2DM in a well-characterized Austrian population sample. To gain some mechanistic insight into the relationship of *SREBF-1* with T2DM, we also explored possible associations of rs2297508 with diabetes-related traits, including β-cell function, insulin sensitivity, lipid status and plasma adiponectin levels.

Methods

Study subjects

We studied 446 unrelated patients with type 2 diabetes recruited from the diabetes outpatient clinics of the Landeskliniken Salzburg and the Krankenhaus Hallein and 1524 glucose tolerant participants of the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) study. Inclusion criteria and laboratory parameters were described elsewhere.[12] Associations of genotypes with estimates of glucose metabolism were determined in 359 glucose tolerant SAPHIR participants, who underwent an oral glucose tolerance test (OGTT).[12] After a 12 h overnight fast, subjects ingested a solution containing 75 g glucose, and plasma samples were obtained at 0, 30, 60 and 120 min for determination of glucose and insulin. Insulin sensitivity/resistance was estimated using the homeostasis model assessment (HOMA) insulin resistance index ($HOMA_{IR}$), the composite insulin sensitivity index ($S_{i(comp)}$), and the metabolic clearance rate (MCR) and S_i proposed by Stumvoll *et al.* as described.[10] β-cell function was estimated by the HOMA-based insulin release index ($HOMA_{Secr}$), first-phase and second-phase insulin release and the CIR_{30} (corrected insulin response at 30 min) as described.[12] All study subjects provided informed consent, and the study was approved by the local ethics committee. The population comprised only white Europeans, mainly of Bavarian and Austrian German descent. Body mass index (BMI) was calculated from measurements of weight and height. Diabetes was diagnosed by fasting plasma glucose concentrations of $\geqslant 7.0$ mmol/l and/or use of hypoglycemic medications.

Laboratory analyses

Venous blood was collected after an overnight fast. Adiponectin was measured using a human adiponectin ELISA kit (BioCat GmbH). Plasma glucose, insulin, cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol and HbA1c levels were measured as described.[10] Genotyping of rs2297508 was performed using restriction fragment length polymorphism (RFLP) detection. 5′-TCCTGTGCTACTTTGC CTTTT-3′ and 5′-GGACAGAGCTGGGAGGTG-3′ were used as forward and reverse primers, respectively, to generate a 370 bp *SREBP-1c* amplicon harboring the rs2297508 polymorphism (GenBank accession number NM_001005291). Optimum polymerase chain reaction (PCR) conditions were achieved with 200 μM dG/dA/dT/dCTP, 1.5 mM $MgCl_2$ and 0.2 U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Primer concentrations were 200 μM each. PCR products were digested with *Xmn*I (New England Biolabs, Beverly, MA, USA) overnight at 37°C before separation of fragments in agarose gels. The accuracy of the RFLP-Assay was verified by dye-terminator sequencing of the respective DNA region in 25 DNA samples. The concordance of 150 RFLP – tested samples performed in duplicate was 100% and all of the subjects enrolled in our study were successfully genotyped.

Statistics

Differences of continuous variables between type 2 diabetic men and women and glucose-tolerant controls as well as effects of genotypes on clinical parameters were ascertained by two-way analysis of variance (ANOVA). Logarithmic transformations were made if the equal variance and normality assumptions of ANOVA were rejected. Measurements were adjusted for effects of age, sex and BMI as indicated. Allele frequencies were estimated by gene counting. Agreement with Hardy–Weinberg expectations was tested using a χ^2 goodness-of-fit test. Differences in genotype frequencies between subjects with T2DM and controls were determined using a χ^2 distribution with two degrees of freedom. To estimate odds ratios (OR) with confidence intervals (95% CI) for each genotype, two 'dummy' variables with the respective wild type as the reference were used in univariate logistic regression analysis. Adjustments for the study subjects' age, sex and BMI were made by including these covariates in a second set of multivariate logistic regression models. To estimate associations of adiponectin plasma levels with indices of insulin resistance and β-cell function, linear models adjusted for age, sex and BMI were used.

Results

Our population-based case–control study included 446 T2DM patients (age of T2DM onset <63 years) and 1524 glucose tolerant SAPHIR participants. Average values for age and BMI were higher and HDL-cholesterol levels were lower in T2DM patients compared to controls (Table 1). As expected from the selection criteria, T2DM patients displayed higher average values for HbA1c. Gender-specific differences were observed for age, BMI cholesterol and HDL cholesterol and a significant interaction between T2DM and gender was observed for BMI.

Genotype frequencies of SNP rs2297508 observed in our control group were similar to values reported in a French population.[10] Hardy–Weinberg expectations were fulfilled in cases and controls. Genotype distributions for SNP rs2297508 differed between subjects with T2DM and controls ($P<0.005$). Estimated unadjusted or adjusted diabetes risks associated with C/G and G/G genotypes were similar, consistent with a dominant effect of the G allele (Table 2). Thus, the C/G and the G/G genotype were associated with a ~1.4-fold higher T2DM risk in our study subjects (Table 2). Gender-specific analysis showed differences of genotype distributions in male subjects ($P<0.0158$), but did not reach significance in female subjects. However, no significant interaction of genotypes and gender was observed, and average age and BMI adjusted OR of C/G (C/G 1.40 (0.98–2.00) vs 1.37 (0.89–2.11); OR (95% CI) male subjects vs female subjects) and G/G subjects (1.43 (0.86–2.37) vs 1.48 [0.83–2.63]) were similar. In the glucose tolerant subgroup who underwent OGTT, no associations of genotypes were observed with indices of insulin resistance and parameters reflecting the insulin secretory response to glucose. Borderline genotype-specific associations with HDL-cholesterol levels were observed in our cross-sectional, non-diabetic cohort, whereas triglycerides, total cholesterol, low-density lipoprotein-cholesterol as well as ApoA1 and ApoB levels were not modified by rs2297508 genotype (Table 3). In the cross-sectional population, 66 out of 1524 subjects received lipid-lowering medication. Genotype distributions did not differ in subgroups with or without drug treatment (data not shown).

In the control population, plasma levels of adiponectin were higher in women than in men and showed an inverse correlation with BMI. Adiponectin levels, adjusted for age, sex and BMI, showed associations with rs2297508 genotype distributions ($P=0.0017$) (Table 4). In the OGTT control group, adiponectin levels were associated with indices of insulin sensitivity/resistance ($S_{i(comp)}$, $P<0.001$, MCR, $P<0.003$; ISI, $P<0.002$), but not with surrogate markers of β-cell function.

Discussion

A role of SREBP-1c in T2DM has been suggested from numerous animal and cell culture models as well as human studies. Our results confirm associations of an exon 18c SNP (rs2297508) with T2DM reported previously in a French diabetic cohort. These studies strongly implicated rs2297508 as a candidate discriminatory site.[10] However, data from the HapMap project (www.hapmap.org) strongly argue for the

Table 1 Clinical characteristics of the study population

Trait	*Diabetic*	*Non-diabetic*	P *disease*	P *sex*	P *interact*
Sex (F/M)	189/257	557/967	—	—	—
Age (years)	61.1±10.6/57.3±9.7	56.0±4.3/49.0±5.4	0.000	(0.000)	(0.000)
Diabetes age at onset (years)	49.0±9.8/49.1±9.0	—	—	—	—
BMI[a] (kg/m^2)	30.4±6.0/28.9±4.9	26.4±4.6/26.6±3.7	0.000	0.009	0.0004
Total cholesterol[b] (mmol/l)	5.33±1.51/5.31±1.15	6.05±1.03/5.90±1.0	NS	0.0000	NS
HDL[b] (mmol/l)	1.46±0.46/1.26±0.46	1.66±0.41/1.38±0.33	0.000	0.000	NS
HbA_{1-c}[c] (%)	8.2±1.8/8.2±1.9	5.4±0.4/5.3±0.4	0.000	NS	NS

Abbreviations: BMI, body mass index; F, female; HDL, high-density lipoprotein; M, male; NS, not significant. Data are means±s.d. [a]Adjusted for age. [b]Adjusted for age, BMI and lipid lowering drugs. [c]Adjusted for age and BMI.

Table 2 rs2297508 genotype and associated risk of type 2 diabetes

	Without type 2 diabetes		*With type 2 diabetes*		P-*value*	*OR (95% CI)*	
	N	%	%	N		*Univariate analysis*	*Multivariate analysis*
Genotype							
CC	616	40.4	31.8	142		1.00	1.00
CG	697	45.7	51.8	231		1.44 (1.14–1.82)	1.37 (1.04–1.81)
GG	211	13.9	16.4	73	0.0045	1.50 (1.09–2.07)	1.45 (0.99–2.11)

Abbreviations: BMI, body mass index; CI, confidence intervals; OR, odds ratios. *P*-values, χ^2 analysis; OR, logistic regression analysis. Multivariate analyses were adjusted for age, sex and BMI.

Table 3 Associations of rs2297508 genotype with phenotypical markers of lipid metabolism in a cross-sectional non-diabetic population

Trait	*CC*	*CG*	*GG*	P *genotype*	P *sex*	P *interact*
Sex (M/F)	389/199	427/238	125/80	NS	—	
Age (years)	50.5±7.6/57.0±6.7	50.8±7.6/57.6±7.0	51.2±7.7/57.2±6.6	NS	—	
BMI[a] (kg/m^2)	27.1±3.9/26.3±4.9	27.3±3.7/26.1±4.4	27.1±3.8/26.7±4.2	NS	0.0054	NS
Cholesterol[b] (mmol/l)	5.90±1.01/6.03±1.07	6.01±1.01/6.06±1.09	6.06±1.07/5.95±0.88	NS	NS	NS
Triglycerides[b] (mmol/l)	1.43±1.01/1.19±0.64	1.54±1.09/1.15±0.55	1.65±1.18/1.18±0.55	NS	0.0001	NS
HDL-Cholesterol[b] (mmol/l)	1.48±0.36/1.79±0.47	1.46±0.34/1.74±0.39	1.46±0.34/1.72±0.44	0.040	0.0001	NS
LDL-Cholesterol[b] (mmol/l)	3.82±0.94/3.72±0.96	3.72±0.91/3.82±1.07	3.90±0.94/3.69±0.81	NS	0.0317	NS
ApoA1[b] (μmol/l)	53.55±8.21/61.05±11.07	53.91±7.85/60.33±9.28	53.55±7.85/59.98±10.71	NS	0.0001	NS
ApoB[b] (μmol/l)	2.09±0.47/2.02±0.44	2.15±0.46/2.06±0.46	2.15±0.47/2.02±0.42	NS	0.0016	NS

Abbreviations: BMI, body mass index; F, female; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, male; NS, not significant. Data are means±s.d.
[a]Adjusted for age. [b]Adjusted for age, sex, BMI and lipid lowering drugs.

Table 4 Associations of rs2297508 genotype with circulating adiponectin levels

Genotype	N	*Adiponectin μg/ml*	P-*value*
CC	607	9.0 (5.6)	
CG	692	8.5 (4.7)	
GG	205	7.9 (6.4)	0.0017

Data are numbers of observations (*N*) or untransformed means (s.d.) adjusted for age, sex and BMI; type 1 and type 2 diabetic subjects are excluded.

existence of a single haplotype block encompassing the entire *SREBF-1* gene including its promoter. Thus, even though rs2297508 might affect mRNA structure and metabolism, additional SNPs within the *SREBF-1* gene promoter, not ascertained yet, might be causative sites by affecting *SREBF-1* gene expression. Interestingly, in mice, a functional variation in the 5′ regulatory region of the *SREBP-1c* gene influenced promoter activity and was shown to be associated with defects in fructose- and insulin-induced hepatic lipogenesis.[13]

In contrast to a previous case–control study including morbidly obese French subjects,[10] no association was observed with BMI in our populations that included slightly overweight subjects only. Furthermore, although previous studies in different obese and other high-cardiovascular risk populations reported associations of rs2297508 with an atherogenic lipid profile,[10,14] we only observed a borderline significant association with HDL-cholesterol levels in our cross-sectional population. This discrepancies may relate to differences in population structure. In a more exploratory approach, we studied possible associations of rs2297508 with diabetes-related traits. Our studies in glucose tolerant subjects failed to identify associations of rs2297508 with indices of insulin sensitivity or β-cell function. Similar results have been reported for the intron 19c SNP that was associated with T2DM in a British population.[11]

However, the association of rs2297508 with plasma adiponectin levels observed in our non-diabetic cohort suggests a potential mechanism that may contribute to the role of the SREBP-1 variants in T2DM. Adiponectin is an adipose-derived plasma protein that is encoded by *ACRP30/ACDC* and hypoadiponectinemia was observed in patients with obesity, insulin resistance and type 2 diabetes.[15] SREBP-1c has been shown to regulate adiponectin gene expression in differentiated mouse adipocytes.[16] Although the human and rodent adiponectin gene promoter reveal only a moderate degree of homology, several putative binding sites for SREBPs are present in the human gene promoter (data not shown). Moreover, *SREBP-1c* mRNA expression is down-regulated in the intra- and extraperitoneal adipose tissue of obese humans, but is restored upon weight loss.[7] Similarly, circulating adiponectin is decreased in obesity,[17] whereas weight reduction results in increased plasma adiponectin.[18,19] Several studies showed associations of circulating adiponectin levels with insulin sensitivity,[15] a result, that was also observed in our control group.

It is thus conceivable that effects on adiponectin gene expression may play a role in the association of the *SREBF-1* locus with T2DM. However, our preliminary results obtained from a cross-sectional population sample should be interpreted with caution and will require confirmation in larger populations.

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